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Innovations

Biolex, Inc.: Pharmaceutical Production in Plants

For decades, agbiotech companies have used the science of genetic engineering and plant-transformation technologies to make more productive, efficient, or desirable plant products. And since 1998, Pittsboro, North Carolina-based Biolex, Inc. has been taking this technology into a new arena: plant-based production of hard-to-make proteins, such as peptides, cytokines, plasma proteins, monoclonal antibodies, and other therapeutic proteins for human health.

"Our mission is to develop human recombinant therapeutic proteins and monoclonal antibodies that until now have been impossible or expensive to develop in existing protein-expression systems," says David G. Spencer, Ph.D., chief operating officer and senior vice-president of R&D at Biolex. He explains that in the more traditional biotech business, protein-expression systems involve suspensions in cell culture—commonly yeast or bacteria such as *E. coli*, or mammalian cells such as Chinese hamster ovary (CHO) cells. "Coming from this tradition, we have been looking for alternative protein-expression systems that can greatly simplify the process," he says. Another traditional way was to use transgenic animals that were genetically engineered to secrete a particular therapeutic protein into their milk. "We have done something similar, except we have used a plant, duckweed, that can be genetically engineered to produce high levels of a therapeutic human protein," he says.

Roots in Forestry Science

The science behind Biolex got its start through the work of Dr. Anne-Marie Stomp at North Carolina State University, Department of Forestry. "She was trying to take the idea of transforming duckweed, a common aquatic weed, and explore how widely it can be used in industry," says Spencer. After securing venture backing for the company, she formed Biolex to take her research into day-to-day practice with the idea of producing industrial enzymes, vaccines, or therapeutic proteins.

> "Though there has been a lot of talk about transgenic animals and plants, most of those platforms have not gone forward because of timing and speed," says David G. Spencer, chief operating officer and senior vice-president of R&D at Biolex. "Ours is the only stable transgenic system that establishes a high-expressing master plant line in six months."

Duckweed, the common name for *Lemna*, is a small, aquatic weed with high protein content and a highly clonal nature that allows it to double in size every 24 to 48 hours. Its rapid growth makes it highly desirable for large-scale production in the contained and controlled environments required for pharmaceutical proteins. "Duckweed has a fortuitous combination of a number of really desirable traits, particularly its rapid clonal growth," says Spencer. "And, it is easily transformed. We get extremely high expression. This combination of features makes duckweed ideal for our mission of transforming biotherapeutics."

Plant cells have an area called the apoplast between the cell wall and membrane. "It is into this space that the duckweed can be induced to secrete the processed protein," says Spencer, explaining that it is possible to control the amount of protein that diffuses from the apoblast into the media surrounding the plant. To recover the protein, one can either process the media or homogenize the plant tissue.

Improved Tissue Yields

Literature on transgenic plants frequently cites results of less than 1% tissue-soluble protein. "In other words, if you homogenize the tissue and recover all of the soluble protein, less than 1% of that would be the desired protein," explains Spencer. "In this optimized duckweed system, we have been able to consistently achieve 7% tissue-soluble protein. That is equivalent to about 10 g/kg dry weight of plant." When expressing a monoclonal antibody, Biolex is able to produce batches of at least 40 g of the antibody in one of its bioprocessing suites every two to four weeks. "So a lot of material can be produced in a short time," he says. "And we have designed different production formats, some of which are suitable for lower volumes, others for very high volume, depending on the desired application."

A number of factors control protein expression level in plants. Some of them are the features of the so-called genetic cassette into which the gene is placed. The cassette includes components such as a highly active promoter to drive the gene into high expression. "The gene also needs to be codon optimized for the plant, meaning you need to have a DNA sequence for the plant's processing equipment," says Spencer. "So you need to have a promoter, a leader sequence, a terminator sequence, and signal peptides, all of which have to work well in concert to generate high levels of expression of the target gene."

Understanding this interplay of genetic components, Biolex sought to improve expression levels early on in its founding. "We started a process of systematically developing a high-expression genetic cassette to produce the proteins we are interested in," says Spencer. "What we did were systematic studies trying a wide variety of these different constructs with the same target protein—just plugging different ones in and out until we got a very high expression cassette. We did this with over 60 different genetic cassettes."

Therapeutic Proteins and Plantibodies

The result is the LEX System, Biolex's program of creating a therapeutic protein from amino acid sequence through scale-up of purified cGMP product suitable for clinical trials and commercialization. The company started by manufacturing alpha interferon and human growth hormone and then progressed to monoclonal antibodies, plasminogen, and other hard-to-make proteins. Alpha interferon is currently made in microbial systems, although Spencer points out that these systems are poorly suited for the job. "One would like to be able to produce it at high expression levels in mammalian cells, but it is a cytokine to which most mammalian cells are sensitive. They have their own receptors for it. As a result, it is very difficult to get high expression." Plants are not sensitive to the cytokine signals of alpha interferon, so it is easy to make them express it at high levels.

The opposite type of problem is monoclonal antibodies where mammalian cells can express them extremely well and at very high levels. The difficulty comes in the product requirements. "If you know that you are going to have to make hundreds of kilograms of protein to satisfy market demand, then the expense of a mammalian-cell culture system—setting that up in terms of capital costs—becomes prohibitive," explains Spencer.

Biolex is pursuing interferon alpha-2b as its lead product. "We have made a product that is even more pure than what is currently in the market," says Spencer. "Throughout the development of our product, we have done head-to-head comparison studies with Intron A, the marketed product, in monkeys, and our phase I clinical trial is also designed that way." Spencer reports that the Biolex product, BLX-883, has the same specific anti-viral and anti-cancer activity as Intron A in assay studies and that all of the in vivo data look comparable in terms of antigenicity, potency, and pharmacokinetics. "Our contaminant levels are extremely low, lower than those reported for the marketed product manufactured via *E. coli* host cell," he adds. The single main hurdle now is to demonstrate in the clinic that the plant-manufactured product presents no unanticipated safety issues and that it is not more antigenic than proteins from any other expression system. The company submitted its first IND to the FDA in December 2004 for BLX-883 and will be initiating a phase I trial immediately. "We will have some of the clinical results later in the first quarter and should have a full report by mid-year," says Spencer. In addition, the compound has already received approval from the Medicines and Healthcare Products Regulatory Agency (MHRA) (the UK health authority) to proceed into phase I testing.

Biolex is also partnering with notable players, such as Centocor, in the protein and monoclonal-antibody fields. Spencer explains that the company assists its partners with the expression of proteins that are either hard to express in other systems or expensive to express in current systems at the required scale. Biolex has initiated two GLP scale-ups with Centocor proteins, and four others are pending. In addition to the monoclonal-antibody programs with Centocor, Biolex also has executed collaborations with Bayer HealthCare, LLC, and Debiopharm S.A.

In May 2004, Biolex acquired Epicyte Pharmaceutical, and with it its Plantibodies technology, the company's intellectual-property-producing antibodies in plants. Patent rights of the technology cover the expression of antibodies and their fragments in plants and protect use of all species of transgenic plants that produce an antibody and methods of making the transgenic plants. Spencer says that acquisition of the Plantibodies patent estate gives Biolex control over plant production of monoclonal antibodies.

Speedy and Self-Contained

The space of transgenic or alternative expression systems has become crowded in the last five to ten years. "Though there has been a lot of talk about transgenic animals and plants, most of those platforms have not gone forward because of timing and speed," says Spencer. For many transgenic plants—as well as transgenic animals—you have to have several generations before you have a commercial-production seed bank or flock. "Ours is the only stable transgenic system that establishes a high-expressing master plant line in six months, which speeds the time to make sufficient product for scale-up and animal and human trials," he says.

The LEX System is contained in facilities designed to ensure safety and lot-to-lot reproducibility. "We are not out in a field, where there is a public perception that spreading of transgenic pollen may contaminate food crops with drugs," says Spencer. Each bioprocessing suite is fully enclosed, contained to approximately 200 square feet. And the plant-based manufacturing system largely avoids the risk of contamination by human pathogens, a limiting factor of human protein production in mammalian cells and transgenic animals.

Biolex has a strong intellectual-property position around the transformation of duckweed and the manufacturing of therapeutic proteins in all plants of the family *Lemnaceae*. "We are the only company that controls transformation of the host and all its subspecies to produce therapeutic proteins," says Spencer. "By comparison, nobody controls transgenic sheep or corn."

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cellpress.com**

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